

Supplemental methods and materials

Generation of yeast strains. To generate gene deletion mutants and for integrative protein tagging we followed the homologous recombination approach described by Longtine *et al*, 1998 and Knop *et al*, 1999, respectively. In brief, cassettes containing a marker gene were amplified by PCR using primers with approximately 50 bases homologous to the flanking regions of the target ORF that was to be modified. The purified PCR products were transformed into yeast cells using a LiOAc/carrier DNA method. Yeast cells were subsequently plated onto selective medium and grown for 3 to 5 days. Single colonies were picked and restreaked onto selective medium. Transformants were then analyzed for their growth behavior on fermentable and non-fermentable carbon sources. Selected yeast colonies were further controlled for gene disruption or marker gene insertion by colony PCR and/or Western-blot analysis.

Co-immunoprecipitation of Coa1^{HA}. After *in vitro* import of mitochondrial precursor proteins, unimported precursors were digested by proteinase K treatment. Mitochondria were reisolated, washed and solubilized in ice-cold lysis buffer for 20 minutes (20 mM Tris, pH 7.4, 60 mM NaCl, 0.5 mM EDTA, 10% glycerol, 1% digitonin). Lysates were subsequently cleared by centrifugation and aliquots of the solubilized extract taken as controls. Next, mouse anti-HA antibody (Roche) was added to the extracts and incubated for 45 minutes on ice. After addition of pre-equilibrated Protein G Sepharose (GE Healthcare), samples were incubated for additional 45 minutes under mild agitation at 4°C. Sepharose beads were washed extensively with lysis buffer containing 0.5% digitonin and bound proteins eluted with 0.1 M glycine pH 3.4. Eluates received SDS sample buffer and were analyzed together with the controls by SDS-PAGE and digital autoradiography.

Membrane potential measurements. Membrane potentials of mitochondria were assessed by fluorescence quenching using the dye DiSC₃(5) (3,3'-dipropylthiadicarbocyanine iodide; Molecular Probes) as described (Geissler *et al*, 2000). Isolated mitochondria (20 µg/ml) were combined with membrane potential buffer (0.6 M sorbitol, 0.1% (wt/vol) bovine serum albumin, 10 mM MgCl₂, 20 mM KPi, pH 7.2), and membrane potentials were dissipated by the addition of 1 µM Valinomycin.

Supplemental references

Geissler A, Krimmer T, Bömer U, Guiard B, Rassow J and Pfanner N (2000)

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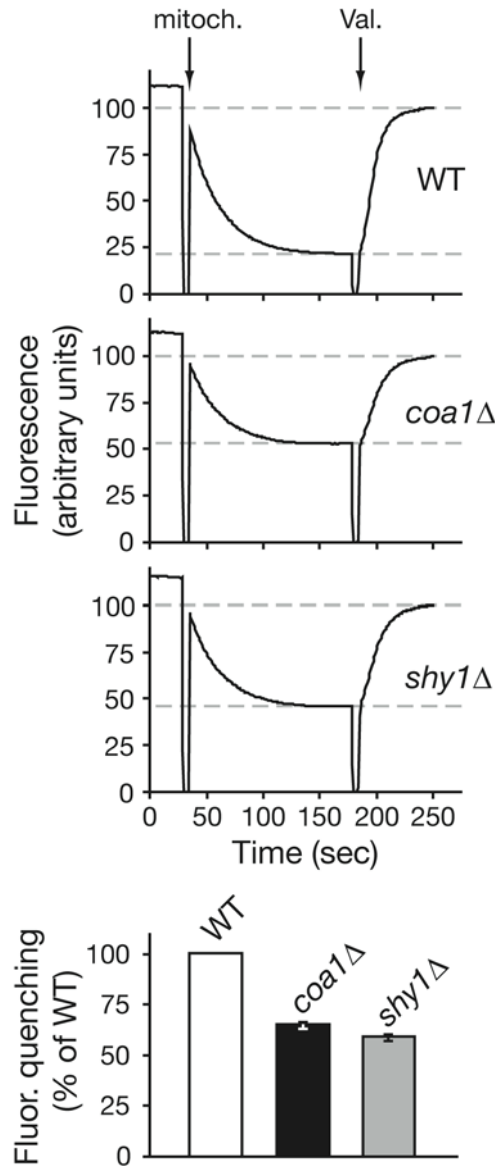
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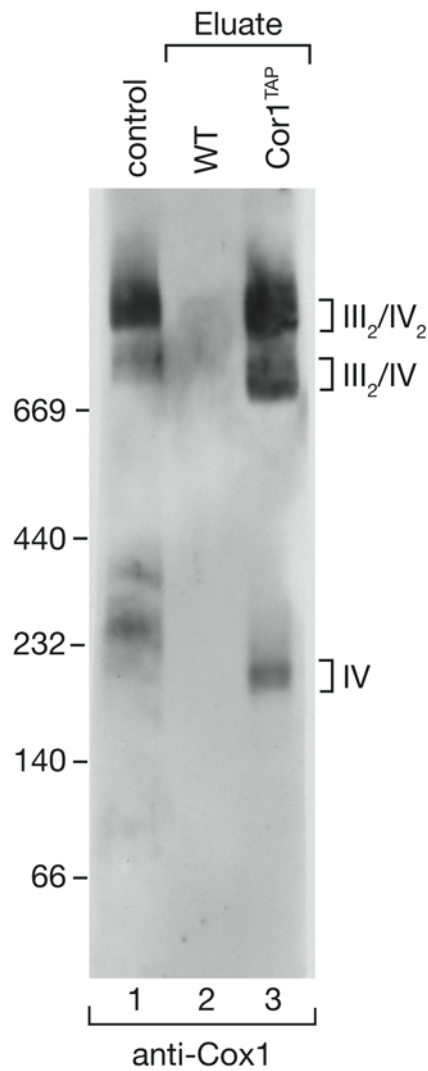
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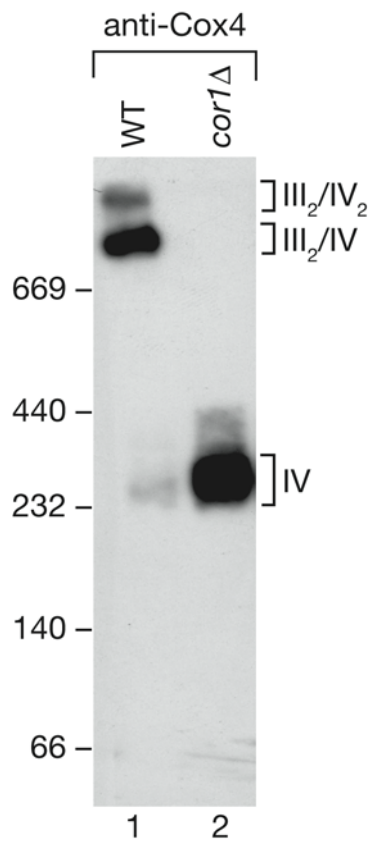
Supplemental data



Supplemental Figure 1. Membrane potential was assessed by fluorescence quenching. Upper three panels, plots of the means of four independent experiments; lower panel, comparative quantification of fluorescence quenching, data represent means \pm s.e.m. (n = 4).



Supplemental Figure 2. Wild-type and Cor1^{TAP} mitochondria were solubilized in 1% digitonin containing buffer and extracts subsequently subjected to IgG-chromatography. After extensive washing, bound material was released from the column by TEV-protease treatment. Wild-type extract (control) and eluates of the IgG-Sepharose were separated by blue-native PAGE, transferred to a PVDF membrane and decorated with anti-Cox1 antiserum. Control, 10%; eluate, 100%. III₂/IV and III₂/IV₂, supercomplexes of complexes III and IV; IV, complex IV monomer.



Supplemental Figure 3. Blue-native PAGE and Western-blot analysis of complex IV in wild-type and *cor1Δ* mutant mitochondria using antibodies against the Cox4 subunit. III₂/IV and III₂/IV₂, supercomplexes of complexes III and IV; IV, complex IV monomer.